Study of immunological effect of *Anastatica hierochuntica* (Kaff Maryam) plant Methanolic extract on albino male mice

دراسة التأثير المناعي للمستخلص الكحولي لنبات كف مريم *Anastatica hierochuntica* على ذكور الفأر الابيض

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Abstract

The objective of this study was to investigate the immunological effect of *Anastatica hierochuntica* extract on albino male mice. The total extract of the raw herb was obtained by using methanol at three different concentrations (25, 50,100)mg/ml. Hydrocortisone used as immunosuppressant drugs (positive control). Adenosine deaminase (ADA) activity, Phagocytosis and immunoglobulin (IgG) were studied with the herbal extracts and appropriate controls. *Anastatica hierochuntica* extract was significantly increasing IgG level when used at dose 50mg/kg (P≤0.05). It also increases significantly the phagocytosis at dose (50,100) mg/kg after 30min and in dose 50 mg/kg after 60 min (P≤0.05). In addition, there was significant inhibition in ADA activity when used plant extract at dose 100 mg/kg after 2 weeks of treatment. Hydrocortisone was found to lead a statistically significant inhibition of phagocyosis, ADA activity, and decrease the IgG level after 2 weeks of treatment (P≤0.05). In conclusion immunomodulatory activity of *Anastatica hierochuntica*, related to IgG level, phagocytosis and ADA activity, is revealed during this study. The herbal extract has shown to promote the IgG level, and also the phagocytosis after 2weeks of treatment.

المستخلص

تهدف هذه الدراسة الى التحري عن التأثير المناعي للمستخلص الكحولي لنبات كف مريم في ذكور الفأر الابيض . تم الحصول على المستخلص الخام لنبات كف مريم باستخدام الميثانول واستخدمت ثلاث جرع (100,50,25)ملغم/كغم . استخدم الهايدروكورتيزون كمثبط مناعي واعتباره سيطرة (سيطرة موجبة) . تمت دراسة تأثير المستخلص النباتي والسيطرة السالبة والموجبة في الفعالية النوعية لأنزيم لادينوسين دي امينيز ، معامل البلعمة ، معدل الكلوبيلين المناعي نوع G . اظهر المستخلص النباتي الخام زيادة معنوية في معدل الكلوبيولين المناعي G عند استخدام الجرعة 50ملغم/كغم وكذلك زيادة معنوية في معامل البلعمة عند الجرعتين (50،100) ملغم/كغم بعد مرور 30 دقيقة وعند الجرعة 50 ملغم/كغم بعد مرور 60 دقيقة (P≤0.05) . بالاضافة الى ذلك اظهر المستخلص النباتي تثبيط معنوي في معدل الفعالية النوعية لانزيم الادينوسين دي امينيز عند الجرعة 100ملغم/كغم بعد مرور اسبوعين من المعاملة (P≤0.05) واظهر عقار الهيدروكورتيزون تثبيط معنوي لكل من معامل البلعمة و الفعالية النوعية لانزيم الادينوسين دي امينيز وانخفاض في معدل الكلوبيلين المناعي نوع G بعد مرور اسبوعين من المعاملة . نستنتج من خلال الدراسة التعديل المناعي لنبات كف مريم والمرتبطة بمعدل الكلوبيولين المناعي نوع G ومعامل البلعمة والفعالية النوعية لانزيم الادينوسين دي امينيز اذ اظهر المستخلص النباتي تحفيز لكل من معدل الكلوبيولين المناعي نوع G ومعامل البلعمة بعد مرور اسبوعين من المعاملة .

Keywords: Adenosine deaminase activity, immunoglobulin, *Anastatica hierochuntica*

Introduction

Herbal medicines are being used by about 80% of the world´s population, primarily in developing countries for routine health care, and also enter the therapeutics in the developed countries [1]. These escape toxicity testing before they are marketed as traditional medicines due to inadequate drug laws. Yet many reports reveal that drugs of plant origin are not free from toxic effects.

Anastatica hierochuntica, belongs to the family Crucifera and the only member of the genus *Anastatica* [2]. A small grey Asiatic desert plant, also known as rose of Jericho. It is a medicinal plant bearing minute white flowers that roll up when dry and expand when moist. This species of medicinal plant is found in Arabic countries, especially in Jordan, Oman, the coast of Egypt, and Libya. In Europe it is known as 'Hand of Fatma' or 'Hand of Maria'. In Malaysia, it is referred as 'Kembang Fatimah' and 'Kaff Maryam' and it has been long used in traditional medicine to facilitate smooth delivery of pregnant women [3], and also used for colds; reduces the pain of and facilitates childbirth; acts as a pain-killer, an emmenagogue, and for epilepsy [4]. Methanolic extracts of *A.* hierochuntica have antioxidant and antimicrobial properties [5], an aqueous extract had a hypoglycaemic effect in both normoglycemic and diabetic rats which was attributed to regeneration and repair of insulin-secreting b-cells [6]. Recent studies include the hepatoprotective activities on D-galactosamine-induced cytotoxicity in cultured mouse hepatocytes at concentrations as low as 3 mol/L. of new skeletal flavonoids, anastatins A and B from *A. hierochuntica* [7], and Antiinflammatory, Anti-melanogenesis activity [8]. The aqueous *A. hirerochuntica* infusions exhibited high antioxidant activity when analyzed by HPLC with an on-line antioxidant detection system [9].

The whole plant contains flavonoids: luteolin-7- glucoside, isovitexin, kaempferol 7-glucoside, kaempferol 3-rhamnoglucoside, quercetin and lucitin. It also contains glucosinolates: glucoiberin and glucocheirolin. The fruits contain glucose, galactose, fructose, sucrose, raffinose and stachyose[8].

Adenosine deaminase an enzyme in the purine catabolic pathway catalyzes the conversion of deoxyadenosine to deoxyinosine and adenosine to inosine. Marked genetic deficiency of ADA has been causally associated with an autosomal recessive form of severe combined immunodeficiency disease (SCID) [10]. Serum IgG levels can be considered as a preliminary yet benign assessment of B-lymphocyte function [11]. Phagocytosis is an important mechanism by which the immune system eliminates pathogens and apoptotic bodies. Successful pathogens have evolved strategies to overcome phagocytosis, including inhibiting internalization, killing or damaging the cell, or by avoiding death inside phagocytes [12].

Aim of study

The aim is to study the effect of *Anastatica hierochuntica* on some immunological parameters include specific activity of adenosine deaminase, phagocytosis and IGg level in serum.

Materials and Methods

Experimental plant

Samples of whole dried *Anastatica hierochuntica* were brought from Kingdom of Saudi Arabia. The aerial parts of the plant were isolated and kept in airtight glass containers till

the time of the experiments. Just prior to experimentation, the dried plant was ground to fine powder.

Extraction of plant materials

Methanolic extract

The plant extracts were prepared using the modified method of [13]. Ten grams of plant dried powder were soaked separately in 100 ml of methanol 98.8%. Then, each mixture was refluxed in water bath in the dark at 45°C. The extracts were filtered through Whatman filter paper No.42. The collected filtrates were dried under vacuum at 40°C using a rotary evaporator, the extraction was repeated twice. The resulting residue was re-dissolved in methanol for used. Three concentrations were prepared for experiment (25, 50,100) mg/kg according to [6].

Hydrocortison solution

The solution was prepared by mix 0.5ml from drug with 49.5ml D.W (50mg/ml). Each mouse administrated 0.066ml (concentration 0.033mg/mice) daily [14].

Experimental animals

Twenty five adult mice for each group weighing (18 – 25)g were used in this study. They were maintained in well ventilated animal house. Animals were housed in large spacious polypropylene cages with free access to food and add libitum during the course of experiment. Male albino mice were orally administered with 3 dose of methanolic extract of plant, once daily for 2 weeks,

- Negative Control mice were orally administered (using a feeding needle to the esophagus) with a daily dose of 0.1 ml distilled water for 2 weeks.
- Plant-treated group: One ml of plant extract (25 mg/kg) was orally administered daily to each mouse in this group for 2 weeks.
- Plant-treated group: One ml of plant extract (50 mg/kg) was orally administered daily to each mouse in this group for 2 weeks.
- Plant-treated group: One ml of plant extract (100 mg/kg) was orally administered daily to each mouse in this group for 2 weeks.
- Positive control (Hydrocortisone solution): 0.066ml of drug was orally administered daily to each mouse in this group for 2 weeks.

Sample collection

Blood was collected from mice by heart puncture; the serum was separated by centrifuge at 5000 rpm for 10min. to use for estimate of IgG level and specific activity of ADA.

Determination of IgG in Mouse Sample

Principle of the assay

The principle of the double antibody sandwich ELISA. In this assay the IgG present in samples reacts with the anti-IgG antibodies which have been adsorbed to the surface to polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-IgG antibodies conjugated with horseradish peroxidase(HRP), are added. These enzyme-labeled antibodies form complex with the previously bound IgG. Following another washing step, the enzyme bound to the immunosorbent is assayed by addition of a chromogenic substrate (TMB). The absorbance, at 450nm, is a measure of the concentration of IgG in the test sample. The quantity of IgG in the test sample can be

interpolated from the standard curve constructed from the standard and corrected from sample dilution.

Phagocytosis Protocol

Principle of the phagocytosis assay is simple according to [15]. Briefly, yeast cells for determining phagocytosis (*Saccharomyces cerevisiae* in this case) are incubated with the macrophages. The cells were stained with Gimsa stain, and attached; engulfed cells were counted with the help of microscope. Calculate percent phagocytosis by counting the number of yeast cells internalized per 100 macrophages.

ADA assay

Assay Principle

The determination of adenosine deaminase activity in the serum was carried out by the method of [16]. This is a colorimetric method based on measurement of the formation of ammonia, which is produced when adenosine deaminase acts on an excess of adenosine. Serum ADA activity was determined at 37ºC according to the method based on the Bertholet reaction, that is, the formation of coloured indophenol complex from ammonia liberated from adenosine and quantified colorimetrically on a spectrophotometer. One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from adenosine at standard assay condition.

Statistical analysis

The results are expressed as their mean± SE. The data were analyzed using one-way analysis of variance (ANOVA), $P \leq 0.05$ was considered significant.

Result

Serum IgG Level:

The present study showed the effect of methanolic extract by a significant increase in the serum IgG level in dose 50mg/kg (471.8 ng/ml) ($P \leq 0.05$) in albino male mice when compared with (-ve) control. In addition to that, it obvious from Table (1) the dose 25mg/kg have no significant decrease in IgG level (230.6 ng/ml) also dose 100mg/kg (299 ng/ml) as compared with (-ve) control ($P \leq 0.05$).The effect of Hydrocortison decrease IgG level (153.2 ng/ml), this decrease was significant when compared with (-ve) control.

Table (1): Serum IgG level in albino male mice given 1 ml daily of methanolic extract of *A. heirocuntica.*

Groups	Dose (mg/kg)	Means ±S.E.	P-Value
Negative control(D.W)	0.0	314.4±10.71a	P≤0.05
Positive control (Hydrocortison)	0.033mg/mice	153.2±24.09b	
First group of mice	25	230.6±10.04ab	
Second group of mice	50	471.8±14.12c	
Third group of mice	100	299±9.11a	

Phagocytosis

Phagocytosis after 30 min.

The result in Table (2) was revealed that the treatment with methanolic extract of plant was significant increase in phagocytosis percentage especially in dose 50mg/kg (37.52%) and 100 mg/kg (34.31%) compared with (-ve) control ($P \leq 0.05$). Although the first dose showed increase in the percentage of phagocytosis (32.69%) but this increase was not significant compared with (-ve) control. As well as the treatment with Hydrocortison

revealed no significant decrease in the phagocytosis percentage reached (25.23%) as compared with (-ve) control (P≤0.05).

Table (2): Phagocytosis percentage after 30 min. in albino male mice given 1 ml daily of methanolic extract of *A. heirocuntica.*

Groups	Dose (mg/kg)	Means(%) ±S.E.	P-Value
Negative control(D.W)	0.0	29.47%±1.21a	P≤0.05
Positive control (Hydrocortison)	0.033mg/mice	25.23%±2.35a	
First group of mice	25	32.69%±0.98ab	
Second group of mice	50	37.52%±1.67b	
Third group of mice	100	34.31%±3.74b	

Phagocytosis after 60 min.

The result in Table (3) was revealed that the treatment with methanolic extract of plant was significant increase in phagocytosis percentage especially in dose 50mg/kg (45.35%) as compared with (-ve) control (P≤0.05). Also the first and third dose showed increase in percentage of phagocytosis (35.26%, 38.41%) respectively but these increase were not significant compared with (-ve) control. On the other hand the treatment with Hydrocortison revealed significant decrease in phagocytosis percentage reached (27.81%) as compared with (-ve) control (P≤0.05).

Table (3): Phagocytosis percentage after 60 min. in albino male mice given 1 ml daily of methanolic extract of *A. heirocuntica.*

Groups	Dose (mg/kg)	Means(%) ±S.E.	P-Value
Negative control(D.W)	0.0	34.91%±1.82a	P≤0.05
Positive control (Hydrocortison)	0.033mg/mice	27.81%±2.03b	
First group of mice	25	35.26%±1.81a	
Second group of mice	50	45.35%±1.97c	
Third group of mice	100	38.41%±4.64a	

Specific activity of ADA

The result of specific activity of ADA was shown in Table (4), the hydrocortisone (0.033mg/Kg) was significant decreased the ADA level reached 2.25IU/mg compared with –ve control. In addition, the third dose (100 mg/Kg) was significantly decreased the ADA level (3.78IU/mg) as compared with (–ve) control. Also no effect was noticed in both first and second dose in ADA level (5.09, 5.27IU/mg respectively) when compared with (–ve) control.

Table (4): Specific activity of ADA in albino male mice given 1 ml daily of methanolic extract of *A. heirocuntica.*

Groups	Dose (mg/kg)	Means(IU/mg) ±S.E.	P-Value
Negative control(D.W)	0.0	5.2±1.04 a	P≤0.05
Positive control (Hydrocortison)	0.033mg/mice	2.25±0.63 b	
First group of mice	25	5.09±0.74 a	
Second group of mice	50	5.27±1.49 a	
Third group of mice	100	3.78±0.66 c	

Discussion

In the present study, the effect of the methanolic extracts of *Anastatica hierochuntica* was tested on albino male mice, it was indicated by the significant increase in IgG level in dose 50 mg/kg that regulation of antibody production induced after 2 weeks of extract plant treatment, this increase in circulating IgG level is believed to contribute to B-cell function, it is believed that the presence of flavone glycosidic components in the plant extract of *Anastatica hierochuntia* [17], is responsible for this increase, this result was

agreement with [18] who found that methanolic extract of *Anastatica hierochuntia* significantly increased the serum levels of IgG, IgA and IgM of diabetic rats. These results are supported with those obtained by [19], they stated that ethanolic extract of *Cleome droserifolia* significantly increased serum total protein, albumin, α-globulin, β-globulin and γ-globulin of rats, the increase may be an expression of the formation of more antibodies.

On the other hand the result showed the negative effect of hydrocortisone by decreasing the titer of IgG in serum, [20] pointed out that the results show marked and significant decrease of IgM concentration in sera of 21- and 42- day-old hydrocortisone-treated rats. Studies done with short-term steroid treatment in humans [21] resulted in a decrease in IgG and IgA, with no significant change in IgM level. [22] Mentioned that bone marrow precursors are resistant to cortison, peripheral B-Iymphocytes are sensitive to it.

In this study it was found that the plant extract presented a potent immunstimulatory effect on functional activity of phagocytic cells in dose (50,100) mg/kg after 30min. and in dose 50 mg/kg after 60 min., these stimulation may be related to presence of some compound like phenolic, flavonoid, β-carotene and lycopene of *Anastatica hierochuntica* that regulate the innate immunity and stimulate the cell to initiate the immune response and accelerating the immune system´s ability to produce T-cell aggregation[5]. It is possible that plant extract can activate the phagocytes and increase the microbicidal activity of these cells which can further improves resistance against infections [23]. Many published reports dealing with the bioactivity of compounds isolated from *A.hierochuntica* gave little information about its antimicrobial activity. The effect of Hydrocortison was clear in significant reduction the percentage of phagocytosis after 60 min. Hydrocortisone has been shown to affect the C3b receptor of both macrophages and neutrophils, whereas the effect on the Fc receptors is controversial, Hydrocortisone treatment of the mice caused a significantly decreased killing only of *Salmonella typhimurium*, although some decrease was also found for *Staphylococcus aureus*, *P. aeruginosa, E. coli, and C. albicans,* hydrocortisone interfered directly with the bactericidal mechanism of the macrophages, this effect could be expected to be the same for all the microorganisms investigated [24]. The existing data also indicates that the plant extract didn't enhance the ADA activity at dose (25, 50) mg/kg, plant extract showed a significant decrease in ADA activity at dose 100mg/kg, ADA is an enzyme capable of catalyzing the catabolism of purine bases (adenosine), and whose principal biologic activity is detected in T-lymphocytes [25], the presence of some compound in plant extract might be convert from positive to negative position when it use in a higher concentration. This result supported with [26] that aqueous extract of *Urtica dioica* leaves because significant inhibition in ADA activities in prostate tissue from prostate cancer patient, this inhibition is dose-depended. At higher amount of extract, ADA is almost inhibition completely.

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